

Reproduction in *Amoeba proteus*

A Dissertation

Submitted to the Board of University Studies of the
Johns Hopkins University in conformity with
the requirements for the degree of
Doctor of Philosophy

By

Percy L. Johnson
Baltimore, Md.

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I. Introduction.

There is marked difference of opinion concerning the characteristics of the organisms designated *Amoeba proteus*. It is generally assumed that the animal RÖSEL VON ROSENHOFF (1755) described is what is ordinarily called *Amoeba proteus*. It is maintained however, by STILES (1905) that according to the International Rules of Zoological Nomenclature, the name *Amoeba proteus* should be *Chaos chaos*. SCHAEFFER (1926) agrees with Stiles that according to these rules the name of RÖSEL'S animal should be *Chaos chaos*, but he contends that RÖSEL'S description is more nearly in accord with that of *Pelomyxa carolinensis* (WILSON, 1900) than with the commonly accepted description of *Amoeba proteus*. SCHAEFFER further holds that the organisms usually designated *Amoeba proteus* consist of three species which he (1917) named *Amoeba proteus*, *Amoeba discoides* and *Amoeba dubia*. In a later paper (1926) he elevates these three species to the rank of genera; *Amoeba proteus* becoming *Chaos diffluens*; *Amoeba discoides*, *Metachaos discoides*; and *Amoeba dubia*, *Polychaos dubia*.

The views held concerning reproduction in these organisms, especially *Amoeba proteus*, are very diverse. This is clearly shown in the following review of the literature on the subject.

CARTER (1863) asserts that when *Amoeba proteus*¹⁾ has attained one-tenth adult size, the nucleus divides by binary fission into two nuclei, and that with subsequent growth these divide again and again until a multinucleate form containing upwards of seventy nuclei is produced (Fig. 1 E). He refers to these nuclei as 'reproductive cells'. He maintains moreover, that when the animals are one-half grown, the 'reproductive cells' are formed at times from chromidia. He did not ascertain the fate of these 'reproductive cells', but assumed that they became 'polymorphic ciliated cells'

¹⁾ Carter designates the organism studied *Amoeba princeps*. Some investigators hold, however, that his description indicated that he had *Amoeba proteus*.

which later developed into amoebae. He postulated the occurrence of 'ovules and spermatozoids' in *Amoeba* and other rhizopods.

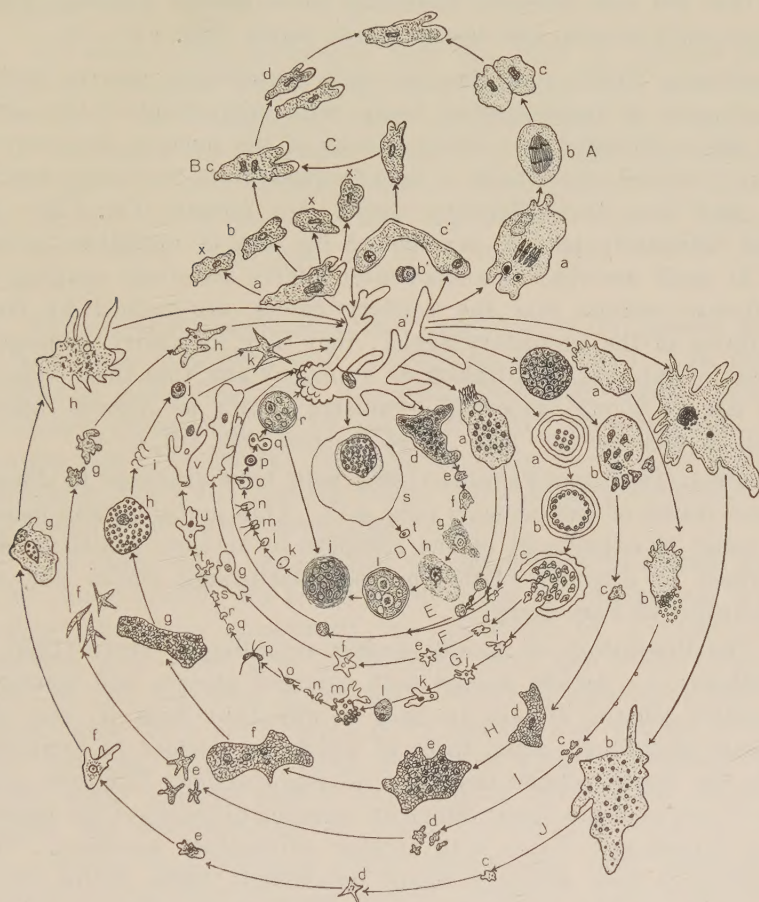


Fig. 1. A diagram representing the reproductive cycle in *Amoeba proteus* as conceived by various investigators. It will be observed that according to these conceptions, the common large amoeba at times gives rise to small amoebae, but that the processes involved differs. A, modified after DOFLEIN (1918); B, from the description of ŠTOLC (1906); C, from the description of ŠTOLC (1906); D, after JONES (1928); E, modified after CARTER (1863); F, modified after METCALF (1910); G, modified after METCALF (1910); H, from CALKINS (1905, 1907); I, modified after HAUSMAN (1920); J, from the descriptions of TAYLOR (1924).

SCHEEL (1899) holds, that at times *Amoeba proteus* encysts by secreting three gelatinous coats around itself (Fig. 1 Fa—c), that during encystment the nucleus undergoes successive direct division

until 500 to 600 daughter nuclei are formed, that each nucleus becomes surrounded by a bit of cytoplasm forming a minute amoeba, and that the cyst disrupts, liberating these minute amoebae which subsequently develop and become large forms (Fig. 1 Fc—h).

CALKINS (1905) accepting SCHEEL's contentions, asserts that a sexual cycle of *Amoeba proteus* begins with encystment of the adult, that this is followed by binary fissions of the nucleus into several 'primary nuclei', that these in turn fragment into 'secondary nuclei', and that from these 'tertiary nuclei' are formed (Fig. 1 Ha—h), which ultimately become surrounded by bits of cytoplasm giving rise to small amoebae. CALKINS later (1907) maintains contrary to his former position that the 'tertiary nuclei' are formed by fragmentation of the 'secondary nuclei', that they in reality undergo a process of internal fertilization or autogamy and that these zygotes give rise to young amoeboid organisms known as *Amoeba radiosa*, which in turn develop into *Amoeba proteus* (Fig. 1 H, h—k).

ŠTOLC (1906) and PRANDTL (1907) both have observed in *Amoeba proteus* fusion of two animals (Fig. 1 C). This probably was merely the result of engulfment of one specimen by another, similar to that described by JENNINGS (1904), or else plastogamic fusion and not conjugation or karyogamy.

The life-history of *Amoeba proteus* according to ŠTOLC (1906) is as follows: An *Amoeba proteus* with enlarged nucleus and increased chromatin content divides in such a way that from it four uninucleate individuals arise, three of which are doomed to extinction while the fourth retains the power of reproduction. This one gives rise to polynucleate forms either by repeated divisions of the nucleus or by cytoplasmic fusion with another uninucleate specimen. This polynucleate form produces again uninucleate forms which either die or retain the power of reproduction (Fig. 1 Ba—d). ŠTOLC draws on the basis of these observations the analogy between this type of reproduction and the formation of polar bodies in metazoan eggs.

METCALF (1910) accepts SCHEEL's contentions, but he asserts that there is a sexual phase in the life-history of *Amoeba proteus*. He holds that small amoebae, liberated from the cyst as described by SCHEEL (1899), at times undergo a process of gemmule formation, and that these gemmules assume a bi-flagellated form, then fuse in pairs, after which the zygotes thus formed develop into *Amoeba proteus* (Fig. 1 Ga—v). He now (1929) thinks that the phenomena

observed were due to parasites in the amoebae and that they consequently do not indicate sexual processes in *Amoeba* (Personal communication).

HAUSMAN (1920) maintains that the body of *Amoeba proteus* sometimes becomes filled with minute nuclei and that this is followed by the extrusion of hyaline spherules from the posterior end with subsequent disintegration of the large animal. He holds that these spherules later become amoeboid and pass successively through the *Amoeba guttula* stage, the *Dactylosphaerium radiosum* stage, the *Amoeba radiosa* stage and finally develop into *Amoeba proteus* (Fig. 1 Ia—h).

TAYLOR (1924) maintains that reproduction in *Amoeba proteus* is sometimes associated with encystment as follows: The nuclear membrane first disappears liberating chromatin blocks into the cytoplasm where each one becomes surrounded by a cyst wall. The amoeba then disintegrates liberating the cysts, which under favorable conditions, hatch and produce minute amoebae later becoming large specimens of *Amoeba proteus* (Fig. 1 Ja—h).

The most recent contribution to reproduction in *Amoeba proteus* is by JONES (1928). He comes to the following conclusions: *Amoeba proteus* undergoes encystment as described by TAYLOR (1924). Amoebae arise from these cysts which grow into large forms and these in turn give rise to secondary nuclei by chromidial formation which later take on a layer of cytoplasm and become gametes. The resulting zygotes encyst, and later produce amoebae which either again undergo gamete formation or develop into organisms having the characteristics of *Amoeba proteus* (Fig. 1 Da'—c', d—t).

HULPIEU and HOPKINS (1927) assert that they demonstrated experimentally that *Amoeba proteus* produces small forms which develop into the large ones. They say (p. 412) they observed, "... in several cultures of large amoebae, (1) that after a period of rapid multiplication by fission the specimens became increasingly more sluggish, darker and more granular in appearance; (2) that they began to decrease in number; and (3) that as the large amoebae decreased small ones appeared and increased". They also placed single specimens in culture fluid on depression slides, and they maintain that these amoebae disappeared and in their place there appeared numerous small amoebae. They also hold that small amoebae were obtained from *Amoeba proteus* under sterile conditions in culture flasks and that these develop into the large

forms. Thus by dealing with single individuals, these investigators seem to have demonstrated that *Amoeba proteus* breaks up into small amoebae which subsequently grow up into the large forms.

If the conclusions presented above are valid it is evident that binary fission is not the only method of reproduction in *Amoeba proteus*, but that reproduction involving gamete formation, sporulation and encystment occurs. It is further evident that the nuclear processes accompanying these diverse modes of reproduction are very different, involving multiple simultaneous nuclear division, repeated nuclear division, fragmentation of the nucleus forming generative chromidia, autogamy and syngamy.

Assuming that the results obtained by HULPIEU and HOPKINS (1927) are valid, I undertook an investigation to ascertain what environmental factors induce fragmentation in *Amoeba proteus*. The methods employed by these investigators appeared to afford a starting point in this study, consequently their experiments were repeated using the methods they employed. This led to a more thorough and comprehensive study of the whole subject of reproduction in *Amoeba proteus*. The results of this study are presented in the following pages.

II. Material and Methods.

The observations to be described in the following pages were made on *Amoeba proteus* derived from the stock used by HULPIEU and HOPKINS (1927). These amoebae retained all characteristics of *Amoeba proteus* (SCHAEFFER, 1917) throughout the entire course of the work. They were cultivated in a number of ways thus affording specimens adapted to various environmental conditions designed to induce different types of reproduction. The usual method of cultivation was as follows: To 100 cc. of spring water (Wyman Park, Chattolane or Tashmoo) in clean finger bowls was added an equal quantity of distilled water, .2 gram timothy hay stems and 1 cc. culture fluid containing *Chilomonas* and other protozoa. The cultures thus prepared were set aside at room temperature, left three or four days and then inoculated with approximately 100 specimens of *Amoeba proteus*. In some of the cultures, wheat was used instead of hay and in still others a modified RINGER solution (HOPKINS, 1928) was used in place of the various spring waters.

The Tashmoo spring water was the best medium found for growing *Amoeba proteus*. The analysis of this water recalculated in

grams per liter is presented in table 1. Cultures prepared with this water were usually acid, those prepared with Chattolane water remained on the alkaline side of neutrality, and those prepared with Wyman Park spring water fluctuated in H-ion concentration from p_H 6.4 to 6.6. The H-ion concentration of the cultures prepared with HOPKINS' solution remained at p_H 6.6.

Table 1.

The Content of Tashmoo Spring Water			
Ammonium chloride	.0000585	grams per liter	
Potassium nitrate	.004108	"	"
Sodium chloride	.064	"	"
Calcium bicarbonate	.0358	"	"
Magnesium bicarbonate	.01527	"	"
Oxide of iron	.0006760	"	"
Silica	.03139	"	"
Sodium sulphate	.0569	"	"
Organic and volatile matter	.05003	"	"

In these cultures it was usually found that the amoebae in two or three weeks decreased in numbers and became inactive. This has been observed by others. The time when it occurs has been called the 'depression period' (TAYLOR, 1924). If flourishing cultures were desired for long periods of time, either subcultures were made as described above or else one-half of the fluid in each old culture was replaced with fresh culture medium.

III. Observations and experimental results.

1. Observations on mass cultures of *Amoeba proteus*.

During four consecutive years several hundred *Amoeba proteus* cultures were prepared as described above and carefully examined at frequent intervals over long periods of time. In one or the other of these cultures, phenomena were observed which are in accord with many of those described by other investigators in support of their conclusions concerning reproduction in *Amoeba proteus*, i. e., the following was observed:

A) In amoebae, having the characteristics of *Amoeba proteus* there was great diversity in form, structure, size, inclusions, number of nuclei and general physiological activity, particularly in cultures in the 'depression periods'. Among these amoebae was the type 'A' of PARSONS (1926), i. e., the type which becomes stellate when put into distilled water and which according to him is likely to undergo

encystment (Fig. 2a). Also there were present in many of the cultures, dark, sluggish individuals like those in which HAUSMAN (1920) and HULPIEU and HOPKINS (1927) assert they obtained fragmentation

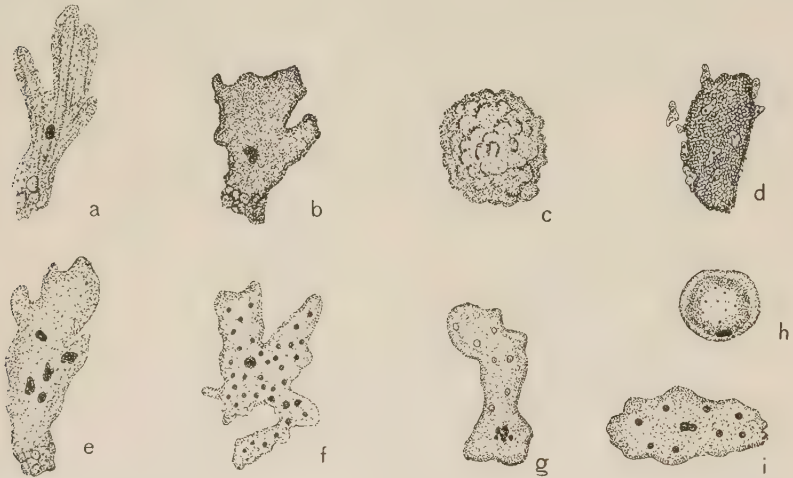


Fig. 2. Camera drawings of specimens of *Amoeba proteus* found in *Amoeba proteus* cultures. a, an amoeba which assumes the stellate form when put into distilled water; b and c, dark sluggish individuals as seen by transmitted light; d, small amoebae appearing to arise from a disintegrated amoeba; e, f and i, amoebae possessing several inclusions resembling nuclei; g, a specimen with a disintegrated nucleus; h, a spherical specimen resembling a cyst. 65:1.



Fig. 3. Camera outlines of *Amoeba proteus* illustrating the great diversity in size (50 to 1200 microns) of different specimens of this species found in some cultures in which food was very scarce. 65:1.

(Fig. 2b and c). There were seen on one occasion in these cultures, small amoeboid forms, seemingly arising from a disintegrated amoeba (Fig. 2d), and on another several amoebae, possessing from forty to sixty rounded inclusions markedly resembling nuclei although smaller (Fig. 2e, f, g and i). These appeared to be multinucleate specimens similar to those described by CARTER (1863) and CALKINS (1905). Specimens without a nucleus were frequently found. These had scattered chromatin indicating that the nucleus had fragmented in accord with the views of some investigators (Fig. 2g). Frequently there were spherical individuals, appearing as though encysted (Fig. 2h). Furthermore it was observed that in these cultures and especially in the old ones, there were specimens of *Amoeba proteus* ranging in length from 50 to 1200 microns. This extensive range in size is demonstrated in the camera outlines presented in Fig. 3. That all of the specimens represented were undoubtedly *Amoeba proteus* is evidenced by the facts that all of them possessed bi-truncated pyramidal crystals and blunt pseudopods, that whenever a nucleus was present it was discoid and that the larger specimens possessed longitudinal ridges on the surface. It will be noted from Fig. 3 that these specimens varied greatly in length and that there was a fairly complete series, a series which compares favorably with that presented by HULPIEU and HOPKINS (1927) in support of their conclusions that the small amoebae develop into *Amoeba proteus*. The small amoebae represented in Fig. 3 did not however originate from the large ones by a so-called fragmentation process as maintained by HULPIEU and HOPKINS, but in the following manner: 1) decrease in volume of large specimens, owing to lack of food and other adverse conditions. 2) nuclear division without subsequent cytoplasmic divisions, resulting in multinucleate forms with three or four nuclei. 3) sloughing off of small portions of the cytoplasm of larger specimens. Small specimens produced by lack of food become large if they are fed, provided starvation has not been carried too far. Large specimens containing several nuclei later may become dissolved into uninucleate ones. These small specimens derived by sloughing off fragments of the large amoebae contain no nuclei and all degenerate without reproduction.

B) A second type of amoeba very much shorter in average length than the specimens which had characters ordinarily ascribed to *Amoeba proteus* occurred in all the mass cultures throughout the whole course of this investigation. The characteristics of these amoebae follow: Size, 30 to 250 microns in length. Form, usually

fan-shaped in culture fluid and stellate in distilled water. Pseudopods, confined mostly to the forward end in locomotion, short, acute and pointed with hyaline substance in them. Surface, smooth. Endoplasm, contains a large number of vacuoles differing in size, many optically active dumb-bell shaped crystals to which are attached little spheres, and three or four contractile vacuoles which open at various places on the surface of the body. Movement, in definite zigzag paths. Rate, about 100 microns per minute. Nucleus, a round vesicle $20 \pm$ microns in diameter, inside of which is a karyosome $11 \pm$ microns in diameter (Fig. 4 a—h)¹. These amoe-

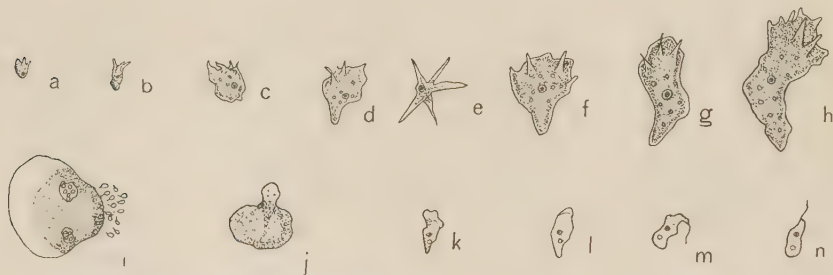


Fig. 4. Camera drawings of amoeboid forms which resemble putative young stages of *Amoeba proteus*. All of these forms were found in *Amoeba proteus* cultures. Range from 30 to 250 microns in length. These specimens taken with those outlined in Fig. 3 form a close series, ranging from the smallest to the largest specimens found; i, amoeboid form giving rise to gametes; j, cyst from which arises an amoeboid form; k, l, *Amoeba guttula* stage; m, n, Amoeboflagellates. a—h, 65:1; i—n, 600:1.

bae resemble figures of putative young *Amoeba proteus* given by HAUSMAN (1920, Pl. I Fig. 14; and Pl. II Fig. 16) and TAYLOR (1927, p. 245, Fig. 1) and also when stimulated they resemble figures representing stages in the life-history of *Amoeba proteus* by SCHEEL (1899, Pl. 51 Fig. 14).

It was repeatedly observed that as *Amoeba proteus* disappeared from the cultures, these small amoebae became very numerous, viz. the results of a typical series of observations. On March 3, 1927 thirteen prolific *Amoeba proteus* cultures were selected, examined and

¹ This type agrees in some respects with the amoebae described by different investigators as distinct species, and especially with specimens described by NERESHEIMER (1905) under the name *Amoeba dofleini* and by SCHAEFFER (1918) under the name *Amoeba bigemma*. For further discussions concerning this matter see JOHNSON (1928).

then set aside. These cultures were kept for a period of two and a half months during which they were examined every two weeks. On March 20 many of the large specimens were sluggish, and on April 2, they had definitely decreased in number and the small forms had become numerous in all the cultures. On May 3 and May 19 the small forms were a little larger than they had been earlier but there was no evidence indicating that they were assuming the characteristics of *Amoeba proteus*. The results obtained in these observations therefore appear to confirm the contentions of TAYLOR (1924) and HULPIEU and HOPKINS (1927) that 'depression periods' are associated with fragmentation of the large amoebae resulting in the production of small ones which in turn develop into large forms. Now the question arises as to whether these small amoebae actually are stages in the life-history of *Amoeba proteus*. This question will be considered in some detail presently.

C) There were found in some of the cultures small amoebae which differ from those described above in that they possessed on the forward end in locomotion a broad sheet of protoplasm (Fig. 4i). These amoebae are similar in some respects to those represented by METCALF in his life-history of *Amoeba proteus* (1910, Fig. 30 and 31).

D) There was also present in the cultures at various times some very small uniflagellate forms about 10 microns long (Fig. 4m and n) which are similar to the flagellate forms described by JONES (1928, Fig. 1). It was observed that they later lost their flagella and assumed the *Amoeba guttula* stage (Fig. 4k and l) and in this condition they resemble forms described by JONES (1928, Fig. 1) and by HAUSMAN (1920, Pl. II, Fig. 11).

E) Small cysts were also present (Fig. 4j) in the cultures at times. These somewhat resembled those described by TAYLOR (1927, p. 60, Fig. 79 and 80). From what has been presented above it is evident that in the *Amoeba proteus* cultures examined there occurred, in reference to size, a fairly complete series of amoeboid forms, ranging from the smallest observed, 10 microns in length, to the largest 1200 microns in length, and that there occurred specimens which were very similar to those described by other investigators as developmental stages in the life-history of *Amoeba proteus*. All these forms thrived under the same conditions as *Amoeba proteus*. There is consequently considerable evidence indicating that they were stages in the life cycle of *Amoeba proteus*. In the following section the question as to whether or not this actually obtains will be considered.

2. Concerning the significance of the small amoebae found in *Amoeba proteus* cultures.

A. Results obtained in an experimental study of mass cultures.

In September 1927, twenty-five *Amoeba proteus* cultures were prepared in finger bowls with Tashmoo spring water as described above. *Amoeba proteus* soon became abundant in all. The finger bowls were then stacked up and left in a room of the Marine Biological Laboratory at Woods Hole until the following summer, after which they were carefully examined with the medium powers of the dissecting binocular and with a water immersion lens on a compound microscope.

Specimens of *Amoeba proteus* were found in seven of the twenty-five cultures, small amoebae in five of these seven cultures and in thirteen others. Five cultures had neither *Amoeba proteus* nor small amoebae. Since large specimens were present in five of the twenty-five cultures it is obvious that they had either persisted over the winter months as such or else had developed from the small amoebae. If they had developed from the small amoebae one would expect large amoebae to develop in some of the other cultures which contained small amoebae only, if the environmental conditions were made favorable. This was attempted as follows:

Five cubic centimeters of the fluid in each of the twenty-five finger bowls was removed and five cubic centimeters fresh sterile hay infusion added. This was repeated each week for over three months. During this period each culture was carefully examined as described above at intervals of about one week. After each examination the H-ion concentration was ascertained by the colorimetric method.

In all the finger bowls food organisms became abundant and a favorable environment for growth was produced and in some amoeboid forms developed. Some of the results obtained concerning these forms are presented in table II. This table shows that on the second examination, specimens of *Amoeba proteus* were found in six of the cultures: and on the third, in ten, i. e., in three new cultures, 2, 14 and 15. This seemed to indicate that some of the small amoebae which were present in these cultures had developed into large ones in accord with the views of HULPIEU and HOPKINS (1927) and TAYLOR (1924). It was observed however, on the first examination,

Table 2.
Date of examination.

Designation of cultures	6/6/28	6/13/28	6/25/28	7/1/28	7/7/28	7/14/28	7/21/28	7/28/28	8/4/28	8/16/28	8/27/28	9/21/28
1	O	O	Ps	Ps	s	s	Ps	s	s	s	O	s
2	s	s	Ps	Ps	s	s	Ps	s	s	s	s	s
3	O	s	s	O	O	O	O	O	O	O	O	O
4	s	s	s	s	O	O	O	s	s	s	s	s
5	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps
6	s	s	s	s	s	s	s	s	O	O	O	O
7	Ps	Ps	Ps	Ps	Ps	Ps	P	Ps	Ps	Ps	Ps	Ps
8	s	s	s	s	s	s	s	s	s	s	s	s
9	s	s	s	O	O	O	O	O	O	O	O	O
10	Ps	Ps	s	O	O	O	P	P	O	s	O	O
11	s	s	s	O	s	s	s	s	s	O	s	s
12	O	O	s	O	O	O	O	O	O	O	O	O
13	Ps	s	s	O	O	O	O	O	O	O	s	O
14	s	s	P	P	s	s	s	P	s	O	O	s
15	s	s	P	s	s	s	s	s	s	O	s	s
16	P	P	P	P	O	O	O	O	O	s	O	O
17	s	s	s	s	O	O	s	O	O	s	s	s
18	P	Ps	Ps	O	O	O	O	s	s	s	s	s
19	s	s	s	O	s	s	O	s	s	s	s	s
20	s	s	s	s	s	s	O	s	s	O	s	s
21	s	s	s	O	s	O	s	s	s	s	s	s
22	s	s	s	s	s	s	s	s	s	s	s	s
23	Ps	P	P	s	s	s	Ps	P	P	P	P	P
24	O	O	s	O	O	O	s	s	s	s	s	s
25	O	O	O	s	s	O	O	O	O	O	O	O

A table showing that the small amoeba found in mass cultures of *Amoeba proteus* do not develop into *Amoeba proteus*. P, *Amoeba proteus* present; s, small amoebae present; O, amoebae absent.

that specimens of *Amoeba proteus* were present in extremely limited numbers and that it was difficult to find these among the algal forms that had arisen in the cultures during the winter months. It therefore appeared probable that large specimens were present at this time and a week later and that they were overlooked in the first two examinations. This contention is supported by the fact that in cultures 1, 2, 10, 14 and 23, *Amoeba proteus* was found in two or three consecutive examinations, then not found for two, three or four consecutive examinations after which it was again found. Moreover, the table shows that no specimens of *Amoeba proteus* were found at any time during the three months of observations in fourteen of the cultures which contained small amoebae, in spite of the fact that the conditions of these cultures were favor-

able for the development of this species¹). There was abundance of food in good condition and the H-ion concentration was favorable, varying but little from p_H 6.8. That the conditions in these cultures were favorable for the development of *Amoeba proteus* is also indicated by the fact that in two of them, 5 and 7, it became especially numerous.

Similar results were obtained in eighty-three other cultures studied for a period of three months and in fifty others studied for a period of five months.

The results in hand therefore strongly indicate that the small amoebae found in cultures of *Amoeba proteus* do not develop into *Amoeba proteus*. It further indicates that once a culture is entirely free from specimens of *Amoeba proteus*, they do not again appear. These conclusions are even more strongly supported by the results obtained in the following experiment.

There was put into a sterile 250 cc. pyrex flask, .2 gram timothy hay stems, 50 cc. distilled water and 50 cc. Wyman Park spring water. The content of the flask was then boiled slowly for ten minutes and allowed to cool after which twelve small amoebae of the type described above (Fig. 4 a—h) were selected, washed in seven changes of distilled water and then put into the flask. The flask was then plugged with sterile cotton and left for two years being however, periodically opened during this interval and a sample poured out and microscopically examined. About fifty different examinations were made during this period. About a month after the flask was prepared, it contained many diatoms and desmids. Large numbers of small amoebae were found in the flask at every examination but no specimens of *Amoeba proteus* were found in any.

The small amoebae remained approximately the same in size throughout the two years, i. e., 30 to 150 microns long and they were in other respects in full accord with the description of small amoebae presented in the preceding section. This seems to show that they are a distinct species. In my opinion (JOHNSON, 1928) they fit the description of an amoeba designated *Amoeba dofleini* by NERESHEIMER (1905) and also the description of an amoeba designated *Mayorella bigemma* by SCHAEFFER (1926). In other words I regard them and the amoebae described by NERESHEIMER and those described by SCHAEFFER, all three animals, as belonging to the same

¹) HULPIEU and HOPKINS (1927) assert that the developmental period of *Amoeba proteus* is about two months. TAYLOR (1924) holds that it may be a year or more.

species and since NERESHEIMER's description preceeds SCHAEFFER's, I shall hereafter call these amoebae *Amoeba dofleini*.

The observations and results presented above indicate that the material on which this investigation was made, originally contained two species of *Amoeba*, *Amoeba proteus* and *Amoeba dofleini*, and it is certain that they were present in the material when it was obtained from HULPIEU and HOPKINS, as evidenced by the fact that an examination of their microscopical preparations agree closely with some of those made in this investigation. Furthermore, a collection of material was made later in Stony Run, the original source of the stock, and there was found not only *Amoeba proteus* but also *Amoeba dofleini*. It appears quite clear then that there was an admixture of species in the original stock of amoebae. However this may be, it is obvious that these small amoebae do not possess the characteristics of *Amoeba proteus* and the evidence presented shows that they do not develop into *Amoeba proteus*. This does not demonstrate however, that there may not be other small amoebae which constitute a portion of the life cycle of *Amoeba proteus*. This matter is considered in detail in the following section.

B. Observations on *Amoeba proteus* in Flask Cultures.

HULPIEU and HOPKINS (1927) maintain that they demonstrated that the small amoebae which appeared in their cultures developed into *Amoeba proteus*. Their experiment was repeated using the same method they used. Four grams timothy hay stems were boiled for ten minutes in two liters of Wyman Park spring water. The solution while still hot was poured into seventeen, sterile, 100 cc. pyrex glass flasks; these were then plugged with sterile cotton. When the flasks were cool a few drops of old culture fluid, filtered through a number 50 Whatman filter paper so as to remove the amoebae but not the *Chilomonas*. *Chilomonas* passed through the filter paper, developed in the solution and served as food for amoebae that were introduced later. One week later, November 20, the flasks were found to contain an abundance of *Chilomonas* but no small amoebae¹). Each of the flasks except two, which were kept for controls, was now inoculated with one specimen of *Amoeba proteus* after it had been washed in six changes of distilled water. On each succeeding day 5 cc. of the fluid was removed and 5 cc.

¹) A similar experiment had been prepared on November 1, but it was discontinued because small amoebae appeared before it was time to inoculate with *Amoeba proteus*. These small amoebae evidently passed through the filter paper.

freshly boiled infusion like the original added. All pipettes used in the experiment were sterilized each time before being used. It is consequently obvious that no amoebae were introduced with this infusion.

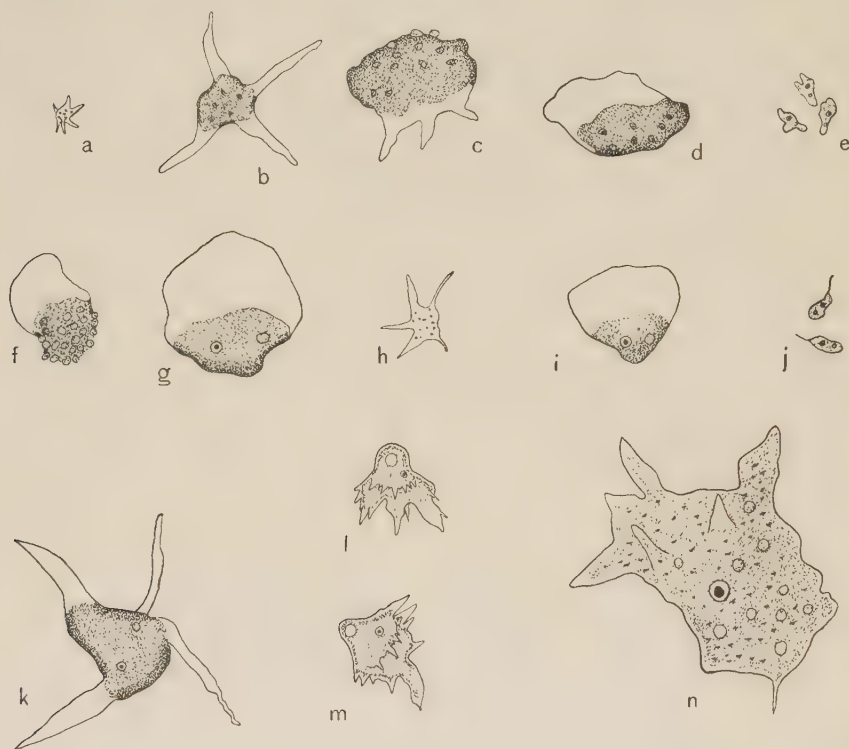


Fig. 5. Camera drawings of small amoebae found in flask cultures, November 1927 to November 1928. a—d, small amoebae found in all the flasks December 1, 1927, ten days after the beginning of the experiment. *Amoeba proteus* also present; f, a new type of amoeboid form which made its appearance February 8, 1928; g—j, forms present in the flasks March 1, 1928 (note the flagellated forms); k, amoeba found June 1, 1928 (note that it is only slightly larger than those found earlier); m, a typical specimen of *Amoeba dofteini*. 600:1.

The contents of the flasks were examined weekly in the following manner: Each flask was shaken by a rotary motion, then the content was poured into a sterile pyrex crystallizing dish. The fluid was given time to settle and then examined, first with the medium powers of the dissecting binocular to detect the presence of *Amoeba proteus*, and then with a compound microscope to which was attached a 1.8 mm. water immersion lens previously sterilized

in 70 per cent alcohol. The results obtained in this experiment are presented in the following description and in figure 5.

On December 1, approximately eleven days after the experiment was started, it was found that *Amoeba proteus* had increased in large numbers and was found in twelve of the seventeen flasks inoculated with *Amoeba proteus*. No specimens of *Amoeba proteus* were found in three of the flasks inoculated and none in the controls. Small amoebae of two kinds were found in all the flasks including the controls. At this time there were therefore three types of amoebae present in the cultures. These differed distinctly in size: *Amoeba proteus* approximately 500 microns long, and two types of small amoebae, one about 10 microns long (Fig. 5a) and another about 30 microns long (Fig. 5b—d). Since the small amoebae were present in the control flasks which did not contain *Amoeba proteus* as well as in those which were inoculated with *Amoeba proteus*, it is evident that they were not produced by the breaking up of the *Amoeba proteus* introduced. However, it seemed advisable to continue the experiment in order to ascertain whether or not the small amoebae found, develop into *Amoeba proteus*.

In the examination of December 24, *Amoeba proteus* was found in only three of the flasks, while specimens of the two types of small amoebae were found in all, and in addition small amoebae of another type, which instead of possessing a number of long slender pseudopods extending in various directions, had during locomotion broad sheets of protoplasm at the anterior end and from twenty to forty spherules at the posterior end (Fig. 5f). When specimens of this type were stimulated they assumed a stellate form with the spherules inside, gradually they began to move by sending out short pseudopods, attachment followed and the spherules gathered at the posterior end (Fig. 5b—d and e). On January 1, *Amoeba proteus* had disappeared in all the flasks, and some of the small amoebae appeared to have crystals attached to spheres. These were, however, not optically active but in spite of this it nevertheless suggested that they were small *Amoeba doylei* (NERESHEIMER, 1905). There were also present at this time in some of the flasks a type of amoeba resembling *Amoeba guttula* (Fig. 5e). On March 1, one culture possessed some uniflagellate forms (Fig. 5j). On April 16, May 9, and June 1, amoebae were found that were slightly larger (Fig. 5g, h) than those found earlier, but they were very much smaller than *Amoeba proteus* and had none of the characteristics of *Amoeba proteus*. After the experiment had been

carried on for over six months, i. e., nearly three times as long as the one made by HULPIEU and HOPKINS, the cultures still contained small amoebae but positively no specimens of *Amoeba proteus*.

All the flasks were now discarded except four, three of which were originally inoculated with *Amoeba proteus* and one which was not. These were kept and examined occasionally. No amoebae having the characteristics of *Amoeba proteus* were found at any time, but on November 16, 1928, a very surprising thing was observed. It was discovered that all four of the flasks had *Amoeba dofleini* in them (Fig. 5n). No amoebae with the characteristics of this species had been found in any of the flasks on any of the numerous previous examinations. These flasks were again examined on January 1, 1929 and again on April 6, 1929. There were still specimens of *Amoeba dofleini* present.

It is obvious that throughout the whole course of this experiment *Amoeba proteus* did not appear in the flasks after it had disappeared. HULPIEU and HOPKINS (1927) maintain that in the one flask they used *Amoeba proteus* completely disappeared in three weeks, and that in eight weeks it was again present, having grown up from small amoebae. In the experiment described above, *Amoeba proteus* peristed in one of the flasks for over seven weeks and the small amoebae found did not develop into *Amoeba proteus* in any of them. This seems to indicate that when HULPIEU and HOPKINS failed to find specimens of *Amoeba proteus* in their flask, three weeks after the beginning of their experiment, they had not entirely disappeared but had decreased in number to such an extent that they were accidentally overlooked and that the numerous specimens of this species found by them later developed from the few that were overlooked and not, as they maintain, from the small amoebae found in the flask.

The question now arises as to whether *Amoeba dofleini* which appeared in the experiment described above, developed from specimens which were accidentally overlooked or from the other types of small amoebae found, and also the question as to the origin of the other types of small amoebae. The former we shall not attempt to answer, but the latter will be considered in the following paragraphs.

Since great precautions were taken to avoid introducing substances of any kind into the flasks after they were prepared, except culture fluid which had passed through the number 50 Whatman filter paper, and small amoebae appeared in every flask, it seemed

likely that they originated from specimens in the culture fluid which passed through the filter paper. In order to ascertain whether or not this obtains, eight *Amoeba proteus* cultures containing small amoebae and the contents of three of the flasks used in the preceding experiment were filtered after which the filtrate was thoroughly examined. Small amoebae were found in every test. This indicated that the small amoebae found in the flasks used in the experiment described above developed from specimens introduced with the filtered culture fluid. If this conclusion is valid no small amoebae should appear if this source of infection is eliminated. In order to test this, the experiment described was repeated as follows:

On March 1, 1929, fourteen flasks were prepared as described above except that in five of them wheat was used instead of hay. Two drops of a sterile culture medium prepared according to PRINGSHEIM (1923, p. 131) was placed on a depression slide, inoculated with a single specimen of *Colpidium* and left for a day. This was then repeated several times taking a *colpidium* from the slide prepared the preceding day. This resulted in a pure culture of *Colpidium*. One specimen from this culture after being washed in several changes of culture fluid was then added to each of the ten flasks prepared. On March 6, 1929, the flasks were examined and found to contain an abundance of *Colpidium* but no small amoebae. The flasks were now inoculated with *Amoeba proteus* as follows: Instead of isolating specimens from a culture as was previously done, one individual was selected, cultured for ten generations on depression slides. The culture medium used was spring water to which specimens of *Colpidium* from a pure culture were added. Twelve or fifteen of the progeny of this amoeba were selected and washed five times in distilled water then one was put into each of the ten flasks. The other four flasks were saved for controls; two of these were inoculated with *Colpidium* only, the other two were not inoculated with anything. The experiment was now continued like the preceding one except that the daily addition of sterile hay and wheat infusion was discontinued after one month.

Up to this time the hydrogen-ion concentration of the cultures remained at approximately p_H 6.6. After the addition of the infusions was discontinued the hydrogen-ion concentration gradually decreased to p_H 7.6. Five of the cultures inoculated with *Amoeba proteus* became prolific *Amoeba proteus* cultures, 200 specimens being repeatedly observed in four drops of fluid taken from the bottom of the cultures. No small amoebae were found in any of the flasks

although they were kept for a longer period than HULPIEU and HOPKINS kept theirs before small amoebae appeared and the hydrogen-ion concentration varied greatly resulting in varied cultural conditions.

The results obtained support the conclusion reached in the preceding experiment; namely, that the small amoebae which appeared in the flasks used in these experiments were introduced through the filter paper, i. e., that they were not produced by the fragmentation of *Amoeba proteus*. The results also indicate that *Amoeba proteus* does not fragment under the conditions of this experiment which were essentially the same as those which obtained in that of HULPIEU and HOPKINS (1927). However, these authors (1927, p. 413) assert that they actually observed fragmentation in *Amoeba proteus* on depression slides. Their observations were therefore repeated as follows:

C. Observations on Individual Specimens of *Amoeba proteus* in Isolation Cultures.

To each of twelve Syracuse watch glasses was added 5 cc. of sterile hay infusion and one large, dark, sluggish specimen of *Amoeba proteus* which had been washed three times in distilled water. The watch glasses were then covered and set aside, after which they were carefully examined periodically under low and high power objectives until after the specimens disappeared. Similar observations were also made on numerous, light, active specimens both on depression slides and in Syracuse watch glasses.

It was found in these tests that the specimens of *Amoeba proteus* disappeared in all of the cultures and that small amoeboid forms appeared coincidently with their disappearance. This seemed to indicate that the small forms arise from the large specimens during the process of disintegration, supporting the view of HULPIEU and HOPKINS (1927). This process of disintegration was, however, repeatedly carefully studied under the microscope, in all its phases and there was nothing observed indicating the formation of the small amoebae from the large ones. The nucleus was always found to be intact in all the different phases and indeed it was the last part to disintegrate, sometimes persisting as long as two weeks after the cytoplasm had disintegrated (Fig. 6i). There however, was observed at this time in one dish both *Amoeba proteus* and small amoebae. This indicated that the large specimen was frag-

menting slowly or else there was contamination. The above experiment was repeated consequently with controls as follows:

Fifteen large, dark, sluggish specimens of *Amoeba proteus* were selected as in the preceding tests, and washed in three changes of distilled water. One was then put into each of twelve watch glasses containing sterile hay infusion. These watch glasses were shaken a bit, after which each amoeba was removed and put into another watch glass containing sterile hay infusion. The first set of watch glasses were kept for control. Observations were made as in the preceding experiment. The results obtained are presented in Table III.



Fig. 6. Camera drawings of stages in the disintegration of large, dark, sluggish specimens of *Amoeba proteus* in isolation cultures. a—g, changes in form that the amoeba passes through; h, cytoplasmic disintegration; i, the nucleus left intact after the cytoplasm disintegrated. The process of disintegration observed was essentially the same in all other specimens. Note the great loss in volume of the specimen. 65:1.

Table 3.
Designation of cultures.

Date of Examination	1	2	3	4	5	6	7	8	9	10	11	12	C
September 1	P	P	P	P	P	P	P	P	P	P	P	P	O
" 4	s	Ps	Ps	s	Ps	Ps	Ps	P	O	Ps	P	O	s
" 7	s	s	s	s	Ps	Ps	s	s	s	Ps	Ps	s	s
" 10	s	s	s	s	s	s	s	s	s	s	s	s	s
" 13	s	s	s	s	s	s	s	s	s	s	s	s	s

Results of observations on individuals of *Amoeba proteus* in isolation cultures. C, controls; P, *Amoeba proteus* present; s, small amoeboid forms present; O, neither found.

It will be seen from this table that the large amoebae disappeared in all the dishes, that small amoeba appeared in all, that

in a number of dishes the small ones disappeared before the large ones disappeared, and that small ones appeared in the controls, which did not contain *Amoeba proteus*, approximately at the same time that they appeared in the cultures containing *Amoeba proteus*. These results seem to prove that the small amoebae found in these cultures did not arise from the disintegration of *Amoeba proteus*.

In some of the tests the watch glasses containing these cultures were kept for more than a month. In some of these all of the small amoeboid forms encysted; in others there was a very surprising occurrence, the small forms became gradually larger and larger until they appeared much like specimens of *Amoeba proteus*, but later it was evident that all had developed into typical mycetozoan plasmodia as indicated by the fact that some of these developed fruiting bodies. It is consequently evident that while some of the amoeboid forms developed into large ones, they did not develop into *Amoeba proteus*¹⁾.

3. Origin of small amoeboid forms found in *Amoeba proteus* cultures.

The experimental results obtained in the foregoing sections indicate that the small amoeboid forms found in *Amoeba proteus* cultures do not arise from *Amoeba proteus* by a so-called fragmentation process and that they do not develop into *Amoeba proteus*. What then is their origin?

A. Hay as a source of amoeboid forms.

In order to ascertain whether or not amoeboid forms arise from hay used in *Amoeba proteus* cultures, nine 100 cc. pyrex glass flasks were sterilized in an autoclave at 20 pounds pressure for forty-five minutes. Five of the flasks while still hot were filled with sterile distilled water and the other four with sterile modified RINGER solution. All were then plugged with sterile cotton. When cooled the plugs were momentarily removed and .2 gram raw timothy hay added to each flask except one, into which was put sterile hay in-

¹⁾ It might be stated here that innumerable attempts were made to induce *Amoeba proteus* on depression slides and in culture dishes, to undergo a process of fragmentation. They were subjected to wide ranges of hydrogen-ion concentration, to varying osmotic pressures, slow dessication, starvation, overfeeding, various salt concentrations, distilled water, different temperatures, and various combinations of these conditions and in no case was there any evidence whatever of any method of reproduction besides fission.

fusion only. This served as a control. All of the flasks were set aside and kept nearly four weeks. During this time they were opened only twice for microscopical examination, December 29 and January 16.

On December 29, five of the culture flasks had amoeboid forms, cysts and flagellates in them, and on January 16 all of the culture flasks, except the control either had or had had these forms. It should be emphasized that the control had none on either date.

The amoeboid forms, cysts and flagellates found in these cultures are represented in Fig. 7a—c, plasmodia differing in size appeared later (Fig. 7d—g), and finally fruiting bodies (Fig. 7h).

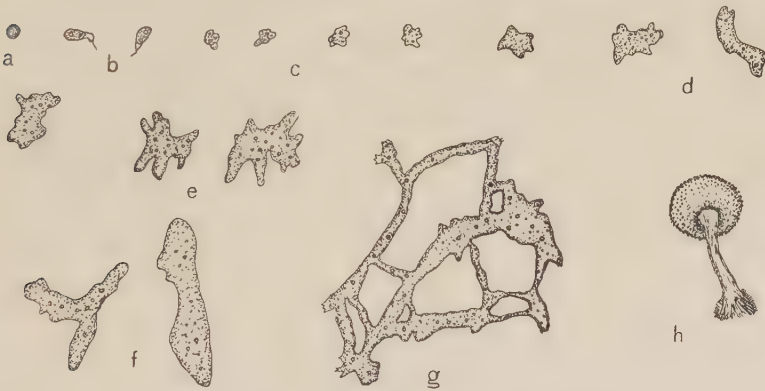


Fig. 7. Camera drawings of stages in the development of Mycetozoa which appeared in hay infusion cultures. a, cyst; b, small Amoeboflagellates; c, small amoeboid forms resembling *Amoeba guttula*; d—g, plasmodia; h, fruiting body. a—c, 600:1; d—g, 65:1; h, 10:1.

In fact all the stages in the life cycle of this species of Mycetozoa, probably *Diderma squamulosum*, were present. The actual emergence of swarmers, their fusion to form the plasmodia and finally the formation of the fruiting bodies was seen to take place. Although similar results were repeatedly obtained using new hay, it should be stated that no such results were obtained with hay three or four years old. The forms obtained in these tests were identical with those which appeared in the experiments in which isolation cultures were used.

These results demonstrate that some of the amoeboid forms, cysts and flagellated forms found in *Amoeba proteus* cultures arise either from the hay used or from the air.

B. Air as a source of amoeboid forms.

In order to ascertain whether or not small amoeboid forms arise from the air or the hay used, tests were made as follows: Twelve 12×70 cm. pyrex glass crystallizing dishes and one 250 cc. pyrex glass flask, each containing modified RINGER salt solution and .2 gram raw timothy hay, were sterilized in an autoclave at twenty pounds pressure for forty-five minutes. The dishes and the flask were then removed and the flask plugged with sterile cotton, but the dishes were left open and exposed to the air of the laboratory. The water level in the dishes was kept constant by adding sterile distilled water. In two weeks there was on the bottom of the dishes many small amoeboid forms, amoeboflagellates and plasmodia, ranging from 10 microns to 2 millimeters in diameter. No trace of amoeboid forms was found in the flask although several examinations were made. It is therefore apparent from this experiment that small amoeboid forms found in *Amoeba proteus* cultures may originate in the air. Further evidence in support of this conclusion is given below.

On January 3, 1928, hay infusion was prepared as usual, by boiling 2 gram timothy hay stems in one liter of spring and distilled water. The solution was then put into ten 250 cc. pyrex glass flasks and autoclaved at twenty pounds pressure for forty-five minutes. Five of the flasks while still hot were plugged with sterile cotton, and the remaining five left open so that the content was exposed to the air of the laboratory. Four days later the contents of one of the plugged flasks and one of the open flasks were microscopically examined and then discarded. The results clearly showed that small amoeboid forms appeared in the open flasks but not in the closed flasks. These must have originated in the air.

It appears very probable that the amoeboid forms get into the air from the timothy hay that was kept in the laboratory. If this is true it is obvious that amoeboid forms found in *Amoeba proteus* cultures may have their origin either in the hay used in the cultures or in the air from spores which have come from the hay.

4. The bearing on the views held concerning reproduction in *Amoeba proteus* of inclusions resembling nuclei.

A. Parasites.

Early in this investigation, while searching cultures for evidence of reproduction in *Amoeba proteus* besides fission, a specimen of *Amoeba proteus* about 800 microns long, was observed to contain from 40 to 50 round bodies similar to nuclei, although smaller

(Fig. 8a). It was later observed that many of the amoebae in this culture, as well as those in two other cultures, possessed similar bodies. It appeared at first that either these were secondary nuclei produced by fragmentation of the original nucleus, or that these specimens were multinucleate amoebae. Critical observations were made on the specimen, illustrated in Fig. 8. Shortly after the observations began on this individual, it divided. Sometimes the round bodies occurred in pairs or fours inside of a membrane, at others they migrated passively in the endoplasmic stream (Fig. 8, 2). One at a time these bodies came to lie at the periphery of the animal and were pinched off with a little cytoplasm (Fig. 8b). Upon being



Fig. 8. Camera drawings illustrating parasitism in *Amoeba proteus*. a, typical large infected specimen (note the encapsulated bodies on the rod-like structures free in the cytoplasm, no nucleus was discernible); b, mode of liberation of bodies; c, mass of disintegrated amoeba; d, liberation of rod-like bodies from the capsules; e, f, rod-like body highly magnified. a—c, 225:1; e, f, 600:1.

separated from the amoebae, feeble amoeboid movements were seen to take place. Shortly after the liberation of these bodies it was observed that they exploded, liberating a large number of rod-like structures, which were prior to their expulsion, undergoing exceedingly rapid movements inside of their capsules. Sometimes these structures were liberated inside of the body of the amoebae. One of these structures, highly magnified, is illustrated in Fig. 8e—f). The amoeba finally disintegrated (Fig. 8e). There was no indication that the rod-like structures conjugated or were associated in any way with reproduction, although the small portions of the cytoplasm which constricted off gave the impression that the amoeba was undergoing fragmentation. In view of the fact that these cytoplasmic fragments containing the nucleus-like bodies, disintegrated soon after being pinched off, it was concluded that the animal was

parasitized. Such infected specimens were never observed again. It is evident then that the form shown earlier in this paper in Fig. 2f, was not a multinucleate specimen of *Amoeba proteus*, but a parasitized one.

B. Water-molds as a source of inclusions.

Specimens of *Amoeba proteus* were frequently found containing six or seven bodies of approximately the same size as the nucleus, and similar in form to the one represented in Fig. 2i. These specimens were at first regarded as multinucleate forms. The cultures containing these had a luxuriant growth of watermold identified by Professor W. C. COKER as *Dictyuchus sterile*. A habit sketch of

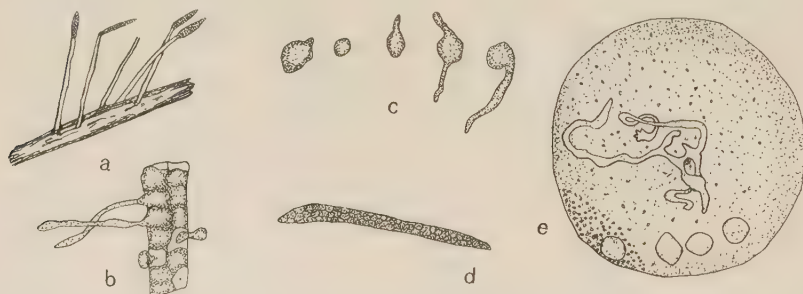


Fig. 9. Camera drawings of water molds in *Amoeba proteus*. a, habit sketch *Dictyuchus sterile*; b, sporangium from branch before shedding spores; c, *Amoeba proteus* with long filament inside; d, development of spores in position; e—h, development of a spore. a, 5:1; b, e, 225:1; c, 125:1; d, 65:1.

this water mold is presented in Fig. 9a. Sporangia develop at the ends of the branches (Fig. 9d), which may break off or germinate in position (Fig. 9d). At other times spores are shed which undergo feeble amoeboid movements and later germinate. It was observed that *Amoeba proteus* engulfs these spores and at times specimens were found containing spores which had sprouted and produced long filaments (Fig. 9e). It is consequently very probable that the form considered in an earlier section of this paper (Fig. 2i) was not a multinucleate specimens of *Amoeba proteus* as implied, but one containing the spores of one of the Saprolegniaceae.

From the results contained in the foregoing sections it is evident that the amoeboid forms found in *Amoeba proteus* cultures originate in various ways. These results indicate that the amoeboid forms and the flagellated forms assumed by various investigators to be stages in the life-history of *Amoeba proteus*, are in no way

genetically related to *Amoeba proteus* and that parasites, other species of *Amoeba*, water-molds and Mycetozoa lead to sources of errors in interpreting reproductive phenomena in *Amoeba proteus*. This conclusion finds support in the views of other investigators as illustrated below.

IV. Views of various other investigators concerning reproduction in *Amoeba proteus*.

Some investigators of amoebae assert that the evidence presented in support of the various methods of reproduction in *Amoeba proteus* given in the introduction of this paper is insufficient.

(SCHAEFFER 1926 a, p. 111) says: "We find no proof that the life cycle of the common large amoeba includes more than . . . reproduction by fission".

KOFOID (1923, p. 401) maintains that the de novo origin of nuclei in *Amoeba proteus* described by CARTER (1915) is the result of misinterpretation.

DOFLEIN (1918, p. 267) in speaking of SCHEELS's work says: "Nun habe ich als junger Student in den Jahren 1897 und 1898 die Entstehung der Arbeit verfolgen dürfen; ja, einige der Abbildungen, welche die Veröffentlichung begleiten, rühren von meiner Hand her. Ich erinnere mich genau an den rotierenden Inhalt der beobachteten Cysten, ich habe die kleinen Amöben, die aus zerfallenden Cysten auskrochen, gesehen".

"Aber es läßt sich nicht mehr beweisen, daß die Cysten wirklich von *A. proteus* herrühren, wie aus dem Amöbenkern die kompakten Kernstränge entstanden und ob wirklich die kleinen Amöben aus den Cysten und dem ursprünglich einheitlichen Amöbenkörper stammten. . . . Aber ob alle Stadien zu *A. proteus* gehören, oder, wie so oft verschiedene Organismen irrtümlich in einen Entwicklungscyclus vereinigt wurden, wage ich nicht zu entscheiden".

The results presented in favor of reproduction in *Amoeba proteus* besides binary fission, have been accounted for by some investigators on the basis of parasitism.

DANGEARD (1895) asserts that the structures in *Amoeba princeps* which CARTER (1863) contends are ovules and spermatozooids, are incontestably the products of parasitism. In general he says, p. 222: "Il est facile de voir, principalement pour les amibes, que toutes ces erreurs sont imputables aux parasites du noyau; on a décrit comme structure du noyau les divers stades de développement des Nucléophages".

PRANDTL (1907) has described *Amoeba proteus* parasitized by a Cryptodiffugian (*Allogromia* sp.). According to him this organism leaves its shell and enters the body of *Amoeba proteus* where it forms a multinucleate plasmodium and there, after a time becomes resolved into gametes which copulate and give rise to an organism with two flagella, and that the zygote thus formed loses its flagella, becomes amoeboid forming a shell like the adult *Allogromia*. He also believes that the observations of CARTER (1863) and those of CALKINS (1904) are to be interpreted on the basis of parasitism.

METCALF (1910, p. 315) says: "It seems . . . not impossible that what CALKINS observed were parasites within the *Amoeba*".

ROBERT (1914, p. 69) writes: „Il s'agit d'une petite espèce rapportée par METCALF à *Amoeba proteus* mais avec quelque doute. Ce cycle a soulevé des doutes parce que même en considérant comme démontré, le bourgeonnement des flagellés, il est possible qu'il s'agisse de parasites qui abandonnent leur hôte pour se conjuguer".

DOFLEIN (1918, p. 257) says in speaking of reproduction in *Amoeba proteus*: „Viele der Beobachtungen sind nicht einwandfrei, beruhen wohl auch auf Verwechslung mit anderen Amöbenarten, auf Kombination verschiedener Formen und auf Nichterkennung von Parasiten der Amöbe".

TAYLOR (1927, p. 244) says of HAUSMAN's work: "I hope to later publish an account of a parasite in *A. proteus* . . . In some cases this parasite exists along with the encysted young in a mature specimen. It is possible that HAUSMAN's material was of this kind".

Furthermore, it is held by some investigators that the affirmed stages in the development of *Amoeba proteus* are in reality different species of *Amoeba* or stages in the life cycle of *Mycetozoa*. HAUSMAN (1920) and TAYLOR (1927) hold that such forms as *Amoeba guttula*, *Dactylosphaerium radiosum* and *Amoeba radiosa* are stages in the life cycle of *Amoeba proteus*. Various taxonomists, however, maintain that all these are distinct species.

MINCHIN (1912, p. 222) says: "METCALF's observations upon syngamy . . . recall strongly the observations of JAHN on the sexual process of of *Mycetozoa*. It is possible that the syngamy observed by him did not form a part of the life cycle of the amoeba, but of some other organism".

DE BARY (1864, p. 87) makes the following statement: "Bei den auf faules Holz, Lohe usw. gemachten Aussaaten traten zwischen den cilienlosen Schwärmern nach einiger Zeit größere amöbenartig bewegliche Körper auf, welche oft von EHRENBURG'S und DUJARDIN'S

Amoeba radiosa, *verrucosa*, *diffluens* nicht oder kaum unterscheidbar waren". And p. 117: "Nichtsdestoweniger liegen einzelne Beobachtungen vor, welche zu der Vermuthung berechtigen, daß manche echte Amöbenformen dennoch dem Entwicklungskreise von Myxomyceten angehören, wenn sie auch in demselben eine andere Stelle als die ihnen früher zugeschriebene einnehmen".

CIENKOWSKI (1876, p. 16) says: "Außer den echten Amöben giebt es noch viele Moneren und nackte Rhizopoden, die durch ihre Beschaffenheit, Bewegungsart, so lebhaft an Myxomycetenplasmodien erinnern, daß sie den Zweifel erwecken, ob sie denn wirklich als selbständige Wesen zu betrachten seien und nicht vielmehr nur abgerissene, herumirrende Plasmodienstücke vorstellen".

DOFLEIN (1901, p. 36) says: "Bis dahin ist aber noch ein weiter Weg, denn die meisten bisher unternommenen Versuche sind daran gescheitert, daß die betreffenden Forscher sie ohne genügende Kenntnis der Amöben und ihrer Biologie unternahmen . . . Darauf weisen die Bilder und nebenbei eingeflochtene Angaben der Autoren hin, so: daß die beobachteten Amöben so sehr zur Cystenbildung neigen, daß sie oft in einen Flagellatenzustand übergehen, bei manchem ist Plasmodienbildung beschrieben (wenn auch oft nicht erkannt): ferner weist das öfter erwähnte Aufwärtskriechen der Kulturen auf Myxomyceten hin".

SCHAEFFER (1926, p. 9) holds: ". . . that some of the very small organisms that have been described as amoebae are really amoeboid stages of myxomycetes, aquatic fungi, etc."

It is consequently evident that according to the views of some investigators the phenomena held by various authors to be reproductive phases in *Amoeba proteus*, as indicated in the introduction of this paper, are associated with parasitism, other species of amoebae and *Mycetozoa*, and consequently are not involved in the reproduction of *Amoeba proteus*.

V. Discussion.

5. What reproductive processes can be justly ascribed to *Amoeba proteus*?

A. Binary fission.

That binary fission is a common method of reproduction in *Amoeba proteus* is well known. Some maintain that it is mitotic and others that it is amitotic. Mitotic division has been observed by AWERINZEW (1904), CALKINS (1905), CARTER (1913), DOFLEIN (1918)

and BĚLAŘ (1926); but some investigators have failed to find any indication of this. TAYLOR (1923) e. g., after a very thorough study of the nucleus failed to find any indication of a mitotic process. However, BĚLAŘ (1926, p. 560) says of TAYLOR'S work: "Die vor kurzem (1923) durch Miss TAYLOR gegebene Schilderung der Teilung von *Amoeba proteus* kann hier wohl unberücksichtigt bleiben, da TAYLOR bestenfalls pathologische Amitosen vorgelegen haben". The positive observations of mitosis in *Amoeba proteus* and the general occurrence of it among other rhizopods, leaves little doubt that it occurs, but it is possible that amitotic division also occurs, especially in old or degenerate specimens.

B. Multiple fission following repeated nuclear division.

The observations of ŠTOLC (1905), SCHAEFFER (1917), DOFLEIN (1918), CARTER (1919), LEVY (1923) and others, show that multinucleate forms of *Amoeba proteus* frequently occur, and that they are produced by repeated or subduplicative divisions of the nucleus, wherein as many as 6 or 8 nuclei are at times produced within a single animal. It is held that the multinucleate individual then becomes dissolved into uninucleate individuals, but not by a coincident, simultaneous segmentation process of the cytoplasm. It is evident that a modification of binary fission occurs in *Amoeba proteus*, i. e., reproduction by plasmotomy, wherein a multinucleate specimen is produced which divides until uninucleate specimens are formed. This method does not however account for the large numbers of small amoebae which are supposed by some investigators to be produced at times.

C. Multiple fission involving multiple nuclear division.

It is held by some investigators that the nucleus of *Amoeba proteus* at times undergoes multiple nuclear division, i. e., that it breaks up by sudden, rapid, direct, sectional divisions into many small nuclei. SCHEEL (1899) maintains that he observed 500 to 600 small nuclei in cysts of *Amoeba proteus* and that these originated by direct multiple nuclear division. Some of these he says were only 3.5 microns in diameter. CARTER (1913) and DOFLEIN (1918) assert they they observed a multipolar spindle in the mitosis in *Amoeba proteus*. HARTMAN regards this as evidence favoring his

polyenergic theory, according to which a large number of nuclei may appear within *Amoeba proteus* through the resolution of a polyenergic nucleus into several monoenergic nuclei. But TAYLOR (1923) regards it as evidence favoring her contention that there is a sporulation process in *Amoeba proteus*. Results obtained by her, later (1924) however, give this contention no support. We may conclude our consideration of this method of nuclei formation with the words of BĚLAŘ (1926, p. 275): "Ein großer Teil dieser Beschreibungen konnte bei nachträglicher Untersuchung nicht verifiziert werden und bei dem heutigen Stand unserer Kenntnisse finden wir diesen Kernteilungsmodus beschränkt auf: Radiolarien, Foraminiferen, Coccidien und *Wagnerella borealis*".

D. Fragmentation and the de novo origin of nuclei.

It is held by some investigators, e. g., CARTER (1863), CALKINS (1905), TAYLOR (1924) and the Hertwigian School, that secondary nuclei sometimes arise in *Amoeba proteus* by chromidia extruded from the nucleus into the cytoplasm by a fragmentation process, and that from these chromidia the nuclei of a new generation of animals are formed. Similar views are held regarding *Arcella*. These views however, do not appear to be well founded, as indicated by the following:

1. PRANDTL (1907) contended that in degenerating specimens of *Amoeba proteus* the nucleus enlarges and becomes hyperchromatic then gives off into the cytoplasm chromidia which degenerate.

2. GRUBER (1882) observed small amoebae in the shell of *Arcella* coincident with the presence of the primary nuclei, but he concluded that these were parasites. DANGEARD (1910) maintains that the contention that secondary nuclei are formed from chromidia, is due to the presence of parasites which were mistaken for nuclei.

3. The work of JOLLOS (unpublished) shows that if the chromidium is digested out with pepsin and trypsin, the primary nuclei still persist, and this appears to be contrary to the views of those who support the chromidial hypothesis; namely, that the primary nuclei disappear when the chromidium appears.

4. REICHENOW (1928) by use of the nucleal reaction was unable to find any evidence of chromatin in the chromidium of *Arcella*. This indicates that the chromidium is not functional in the production of nuclei. It has been suggested that it plays a part in the formation of the test.

5. WILSON (1925, p. 597) in speaking of the chromidial formation of nuclei in general says: "None of these accounts seem to the writer to be sufficiently demonstrated, and they have in fact been viewed with considerable skepticism by specialists in this field. In any case, all call for the most careful re-examination".

6. BĚLAŘ (1926, p. 527) says in speaking of the chromidial hypothesis: "Und was für diese gilt, das gilt auch für die Fortpflanzungsphänomene, die sich am Chromidium abspielen sollen; auch hier gleicht keine Darstellung ganz der andern, auch hier ist die Fehlerquelle einer Infektion durch Parasiten nirgends ausgeschaltet, nirgends durch N. B. genau kontrollierte!").

E. Encystment in *Amoeba proteus*.

SHEEL (1899), CARTER (1915) maintain, as previously stated, that *Amoeba proteus* encysts by secreting the gelatinous coats about itself and that numerous nuclei are formed during encystment. SHEEL contends that the nuclei are formed by repeated division of the nucleus, while CARTER contends that they are formed from chromidia. TAYLOR holds that minute cysts are formed inside the *Amoeba* and that the nuclei of the cysts are formed from chromidia. The fact that these views differ so radically indicates that the evidence in favor of reproductive cysts is not conclusive. Moreover, many investigators hold that *Amoeba proteus* does not encyst.

F. Conjugation, autogamy, gamete formation and syngamy in *Amoeba proteus*.

The conflicting views regarding these processes in *Amoeba proteus* prohibit one from accepting any as definitely proved. In fact these processes and those mentioned above all call for a re-examination.

VI. Summary and Conclusions.

There is great diversity of opinion concerning reproduction in *Amoeba proteus*. All investigators hold that it reproduces by binary fission; but it is maintained by some that *Amoeba proteus* in one way or another, at times gives rise to small amoebae and that these develop into large ones. The processes held to obtain in the formation of the small amoebae differ greatly however, comprising mitotic division, amitotic division, repeated nuclear divisions, multiple nuclear division, chromidial formation of nuclei, agamete

formation, gamete formation, fertilization, autogamy, plasmogamy, plasmotomy, encystment, sporulation, gemmation and a sort of viviparous reproduction.

In this investigation, phenomena were observed which are in accord with many of those described by other investigators in support of their conclusions concerning reproduction in *Amoeba proteus*. They are as follows:

1. In mass cultures of *Amoeba proteus*, flagellated forms and a fairly complete series of amoebae, ranging in size from 10 to 1200 microns in length were found, i. e., forms similar to those represented by other investigators as putative young stages of *Amoeba proteus*.

2. Some of the small amoebae found possessed the characteristics of *Amoeba proteus* and are produced by great loss in volume of large specimens owing to starvation and adverse conditions, and by cutting off cytoplasmic fragments without nuclei. Others are *Amoeba dofleini* and still others, including the flagellate forms, are developmental stages of *Mycetozoa*.

3. There was no indication that any of these small amoebae develop into *Amoeba proteus*, except those which resulted from a decrease in volume of the large specimens owing to lack of food.

4. Mass cultures completely depleted of *Amoeba proteus* never afterwards yield *Amoeba proteus*.

5. Specimens of *Amoeba proteus* isolated in flasks, Syracuse watch glasses and depression slides, disappear, and numerous minute amoeboid forms frequently appear.

6. These small amoebae however, are not produced by *Amoeba proteus* and they never develop into *Amoeba proteus*. They either remain small or develop into *Mycetozoa*.

7. Parasites and aquatic fungi in *Amoeba proteus* lead to misinterpretations.

8. The results of this investigation indicate that binary fission in reference to the nucleus, is the only method of reproduction in *Amoeba proteus*. However, sometimes the cytoplasm does not divide when the nucleus does and this results in multinucleate forms containing sometimes as many as six nuclei. These individuals may then divide by multiple cytoplasmic fission.

9. These conclusions are supported by the views of several other investigators.

Literature cited.

- AWERINZEW, S. (1904): Über die Teilung bei *Amoeba proteus* PALL. Zool. Anz. Bd. 27 p. 399—400.
- BĚLAŠ, K. (1926): Der Formwechsel der Protistenkerne. Ergebn. u. Fortschr. d. Zool. Bd. 6 p. 235—654.
- CALKINS, G. N. (1905): Evidences of a sexual-cycle in the life-history of *Amoeba proteus*. Arch. f. Protistenk. Bd. 5 p. 1—16.
- (1907): The fertilization of *Amoeba proteus*. Biol. Bull. Vol. 13 p. 219—230.
- CARTER, H. J. (1856): Notes on the fresh water infusoria of the island of Bombay. Ann. and Mag. Nat. Hist. Vol. 18 p. 115—132.
- (1856): Notes on the fresh water infusoria of the island of Bombay. Ibid. Vol. 18. p. 221—249.
- (1863): *Amoeba princeps* and its reproductive cells, compared with *Aethalium*, *Pythium*, *Mucor* and *Achlya*. Ibid. Vol. 12 Ser. 3 p. 30—52.
- CARTER, L. A. (1913): Note on a case of mitotic division in *Amoeba proteus* PALL. Proc. Roy. Phys. Soc. Edin. Vol. 19 p. 54—59.
- (1919): Some observations on *A. proteus*. Ibid. Vol. 20 p. 193—210.
- (1915): The cyst of *Amoeba proteus*. Ibid. Vol. 19 p. 204—212.
- CIENKOWSKI, L. (1876): Über einige Rhizopoden und verwandte Organismen. Arch. f. Mikr. Anat. Bd. 12 p. 15—50.
- COKER, W. C. (1923): The Saprolegniaceae. North Carolina 201 p.
- DANGEARD, P. (1895): Mémoire sur les parasites du noyau et du protoplasma. Le Botaniste T. 4 p. 199—248.
- (1910): Études sur le développement et la structure des organismes inférieures. Ibid. T. 11 p. 1—311.
- DE BARY, A. (1864): Die Mycetozoen. 132 S. Leipzig.
- DOFLEIN, F. (1901): Die Protozoen als Parasiten und Krankheitserreger. 274 S. Jena.
- (1918): Die vegetative Fortpflanzung von *A. proteus* PALL. Zool. Anz. Bd. 49 p. 257—268.
- DOFLEIN und REICHENOW, E. (1927—1928): Lehrbuch der Protozoenkunde Bd. 1 u. 2 864 S.
- GRUBER, A. (1882): Die Theilung der monothalamen Rhizopoden. Zeitschr. f. Wiss. Zool. Bd. 36 p. 104—124.
- HAUSMAN, L. A. (1920): A contribution to the life history of *Amoeba proteus* LEIDY. Biol. Bull. Vol. 38 p. 340—352.
- HOPKINS, D. L. (1928): The effects of certain physical and chemical factors on locomotion and other life processes in *Amoeba proteus*. Journ. Morph. and Physiol. Vol. 45 p. 97—119.
- HOPKINS, D. L. and JOHNSON, P. L. (1929): The culture of *Amoeba proteus* in a known salt solution. Biol. Bull. Vol. 56 p. 68—73.
- HULPIEU, H. R. and HOPKINS, D. L. (1927): Observations on the life-history of *Amoeba proteus*. Biol. Bull. Vol. 52 p. 411—417.
- JENNINGS, H. S. (1904): Contributions to the study of the behavior of lower organisms. Pub. Carn. Inst. Wash. 257 p.
- JOHNSON, P. L. (1928): *Amoeba dofleini* (NERESHEIMER) vs. *Mayorella bigemma* (SCHAEFFER). A case of synonymy. Science Vol. 68 p. 84—85.

- JONES, P. M. (1928): Life cycle of *Amoeba proteus* (*Chaos diffluens*) with special reference to the sexual stage. Arch. f. Protistenk. Bd. 63 p. 322—332.
- KOFORD, C. A. (1920): A critical discussion of the chromidial formation of nuclei, of autogamy, and of multiple division of polyenergic nuclei, with a special reference to the unity of the mechanism of heredity in the Protozoa and Metazoa. Anat. Rec. Vol. 20 p. 223—225.
- (1923): The life cycle of the Protozoa. Science Vol. 62 p. 397—408.
- LEVY, J. (1924): Studies on reproduction in *Amoeba proteus*. Genetics Vol. 9 p. 124—150.
- LISTER, A. (1894): Mycetozoa. 224 p. London.
- MACBRIDE, T. H. (1922): The North American Slime-moulds. 347 p. New York.
- METCALF, M. M. (1910): Studies upon *Amoeba*. Journ. Exp. Zool. Vol. 9 p. 301—331.
- MINCHIN, E. A. (1912): An introduction to the study of the protozoa. 520 p. London.
- NERESHEIMER, E. (1905): Über vegetative Kernveränderungen bei *Amoeba dohleini*. Nov. sp. Arch. f. Protistenk. Bd. 6 p. 147—165.
- PARSONS, C. W. (1926): Some observations on the behavior of *Amoeba proteus*. Quart. Jour. Mic. Sci. Vol. 70 p. 629—646.
- PRANDTL, H. (1907): Die physiologische Degeneration der *Amoeba proteus*. Arch. f. Protistenk. Bd. 8 p. 281—293.
- (1907a): Der Entwicklungskreis von *Allogromia* sp. Arch. f. Protistenk. Bd. 9 p. 1—21.
- PRINGSHEIM, E. G. (1923): Zur Physiologie saprophytischer Flagellaten. Beitr. z. allg. Bot. Bd. 2 p. 88—137.
- REICHENOW, E. (1928): Ergebnisse mit der Nuclealfärbung bei Protozoen. Arch. f. Protistenk. Bd. 61 p. 144—166.
- ROBERT, A. (1914): Conférences de Zoologie faites à la Sorbonne. 450 p. Paris.
- RÖSEL VON ROSENHOFF, A. J. (1755): Der kleine Proteus. Der Insecten-Belustigung. Bd. 3 p. 622—624. Nuremberg.
- SCHAEFFER, A. A. (1917): Notes on the specific and other characters of *Amoeba proteus* PALLAS (LEIDY). *A. discoides*, spec. nov. and *A. dubia*, spec. nov. Arch. f. Protistenk. Bd. 37 p. 204—228.
- (1918): Three new species of amebas; *Amoeba bigemma* nov. spec., *Pelomyxa lentissima* nov. spec., and *P. Schiedti* nov. spec., Trans. Am. Mic. Soc. Vol. 37 p. 79—96.
- (1926): The taxonomy of the amebas. Pub. Carn. Inst. Wash. Vol. 24 No. 345 p. 1—116.
- (1926a): Recent discoveries in the biology of amoeba. Quart. Rev. of Biol. Vol. 1 p. 95—118.
- (1928): Specific differences between the amebas *Mayorella bigemma* and *M. (?) dohleini*. Am. Nat. Vol. 63 p. 88—94.
- SHEEL, C. (1899): Beiträge zur Fortpflanzung der Amöben. Festschr. zum 70. Geburtstag von CARL VON KUPFFER p. 569—580.
- SCHIRCH, P. (1914): Beiträge zur Kenntnis des Lebenscyclus von *Arcella vulgaris* EHRBG. und *Pelomyxa palustris* GREEFF. Arch. f. Protistenk. Bd. 33 p. 247—271.

- STILES, C. W. (1905): Public health papers and reports. Am. Pub. Health Assoc. Vol. 30 p. 293.
- ŠTOLC, A. (1905) Über die Teilung des Protoplasmas im mehrkernigen Zustande. Arch. f. Entwicklungsmech. der Org. Bd. 19 p. 631—647.
- (1906): Plasmodiogenie, eine Vermehrungsart der niedersten Protozoen. Ibid. Bd. 21 p. 111—125.
- TAYLOR, M. (1923): Nuclear divisions in *Amoeba proteus*. Quart. Journ. Mic. Sci. Vol. 67 p. 39—46.
- (1924): *Amoeba proteus*: Some observations on its nucleus, life-history and culture. Ibid. Vol. 69 p. 119—143.
- (1927): The development of the nucleus of *Amoeba proteus* PALLAS (LEIDY) (*Chaos diffluens* SCHAEFFER). Ibid. Vol. 71 p. 239—258.
- WILSON, E. B. (1925): The cell. 1232 p. New York.
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